

U.S. Patent Application Serial No. **09/340,196**
Amendment filed April 27, 2005
Reply to OA dated December 30, 2004

AMENDMENTS TO THE CLAIMS:

Please cancel claims 51, 53, 54 and 56 without prejudice or disclaimer. Please amend claims 59 and 68 to 77, and add new claim 78, as follows. This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1-58 (Canceled).

Claim 59 (Currently amended): A method for determining malignancy of a thyroid tumor comprising:

(1) measuring an amount of one of two types of thyroglobulin in a fluid sample originating from a living body, the steps comprising:

(a) adding to the sample a specific lectin or a specific antibody capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin, to form a conjugate of the specific lectin or the specific antibody with the first type of thyroglobulin;

(b) separating said conjugate from the non-conjugated second type of thyroglobulin;

(c) measuring said conjugate content by adding a first anti-thyroglobulin antibody capable of binding to both types of the thyroglobulin, for determining the amount of the first type of thyroglobulin; or

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(d) measuring an amount of the non-conjugated second type of thyroglobulin by adding a first anti-thyroglobulin antibody capable of binding to both types of the thyroglobulin,

(2) determining malignancy of the thyroid tumor by comparing a calculated ratio of the amount measured in (c) or (d) to an amount of total thyroglobulin in the sample with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant in any of the following cases (i) to (iv),

(i) when the calculated ratio is significantly higher ~~or lower~~ than that of the reference fluid sample of the normal thyroid and is significantly higher ~~or lower~~ than that of the reference fluid sample of the benign thyroid,

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid,

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, or

(iv) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid.

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Claims 60 - 67 (Canceled).

Claim 68 (Currently amended): A method for determining malignancy of a thyroid tumor comprising the steps of:

- (a) adding to a fluid sample originating from a living body:
 - (i) a specific lectin or a specific antibody capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin, and
 - (ii) a first anti-thyroglobulin antibody capable of binding to both types of the thyroglobulin, to form a first conjugate which is a conjugate of the first anti-thyroglobulin antibody with the first type of thyroglobulin and with the specific lectin or the specific antibody, and a second conjugate which is a conjugate of the first anti-thyroglobulin antibody with the second type of thyroglobulin;
- (b) measuring an amount of the first type of thyroglobulin on the basis of the first conjugate content; and
- (c) measuring an amount of the second type of thyroglobulin on the basis of the second conjugate content;
- (d) calculating a ratio of ~~(a)~~ the amount of the first type of thyroglobulin measured in (b) to the amount of total thyroglobulin; or ~~(b)~~ the amount of second type of thyroglobulin measured in

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(c) to the amount of total thyroglobulin; and

(e) determining the malignancy of a thyroid tumor by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant in any of the following cases (i) to (iv),

(i) when the calculated ratio is significantly higher ~~or lower~~ than that of the reference fluid sample of the normal thyroid and is significantly higher ~~or lower~~ than that of the reference fluid sample of the benign thyroid,

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid,

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, or

(iv) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid.

Claim 69 (Currently amended): A method for determining malignancy of a thyroid tumor

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comprising the steps of:

(a) adding to a fluid sample originating from a living body, a specific lectin or a specific antibody capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin,

to form a conjugate of the specific lectin or the specific antibody with the first type of thyroglobulin;

(b) separating the conjugate from the second type of thyroglobulin; and

(c) measuring an amount of the first type of thyroglobulin on the basis of the conjugate content by adding a first anti-thyroglobulin antibody capable of binding to both types of the thyroglobulin; and

(d) measuring an amount of the separated second type of thyroglobulin by adding a first anti-thyroglobulin antibody capable of binding to both types of the thyroglobulin;

(e) calculating a ratio of ~~(a)~~ the amount of the first type of thyroglobulin measured in (c) to the amount of total thyroglobulin; or ~~(b)~~ the amount of second type of thyroglobulin measured in (d) to the amount of total thyroglobulin; and

(f) determining the malignancy of a thyroid tumor by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant in any of the following cases (i) to (iv),

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(i) when the calculated ratio is significantly higher ~~or lower~~ than that of the reference fluid sample of the normal thyroid and is significantly higher ~~or lower~~ than that of the reference fluid sample of the benign thyroid,

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid.

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, or

(iv) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid.

Claim 70 (Currently amended): A method for determining malignancy of a thyroid tumor comprising the steps of:

- (a) adding to a fluid sample originating from a living body:
 - (i) a specific lectin or a specific antibody capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin,
 - (ii) a first anti-thyroglobulin antibody capable of binding to both the first type of

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thyroglobulin and the second type of thyroglobulin, and

- (iii) a second anti-thyroglobulin antibody capable of binding to the two types of thyroglobulin, but not capable of binding to the thyroglobulin to which the specific lectin or the specific antibody is already bound,

to form a first conjugate which is a conjugate of the first anti-thyroglobulin antibody with the first type of thyroglobulin and with the specific lectin or the specific antibody, and a second conjugate which is a conjugate of the first anti-thyroglobulin antibody with the second type of thyroglobulin and the second anti-thyroglobulin antibody;

- (b) separating the first conjugate and the second conjugate; and

- (c) measuring an amount of the first type of thyroglobulin on the basis of the first conjugate content; and

- (d) measuring an amount of the second type of thyroglobulin on the basis of the second conjugate content;

- (e) calculating a ratio of ~~(a)~~ the amount of the first type of thyroglobulin measured in (c) to the amount of total thyroglobulin; or ~~(b)~~ the amount of second type of thyroglobulin measured in (d) to the amount of total thyroglobulin; and

- (f) determining the malignancy of a thyroid tumor by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

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wherein the sample is determined to be malignant in any of the following cases (i) to (iv).

(i) when the calculated ratio is significantly higher ~~or lower~~ than that of the reference fluid sample of the normal thyroid and is significantly higher ~~or lower~~ than that of the reference fluid sample of the benign thyroid,

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid,

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, or

(iv) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid.

Claim 71 (Currently amended): A method for determining malignancy of a thyroid tumor comprising the steps of:

- (a) adding to a sample originating from a living body:
 - (i) a specific lectin or a specific antibody capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin, and

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- (ii) an anti-thyroglobulin antibody-2 capable of binding to the two types of thyroglobulin, but not capable of binding to the thyroglobulin to which the specific lectin or the specific antibody is already bound,

to form a first conjugate which is a conjugate of the specific lectin or the specific antibody with the first type of thyroglobulin, and a second conjugate which is a conjugate of the anti-thyroglobulin antibody-2 with the second type of thyroglobulin;

- (b) separating the first conjugate and the second conjugate formed in the step (a);

(c) adding an anti-thyroglobulin antibody-1 capable of binding to both types of thyroglobulin to the second conjugate formed in the step (a), to form a third conjugate which is a conjugate of the anti-thyroglobulin antibody-2 with the second type of thyroglobulin and with the anti-thyroglobulin antibody-1;

(d) measuring an amount of the first type of thyroglobulin on the basis of the first conjugate content and

(e) measuring an amount of the second type of thyroglobulin on the basis of the third conjugate content;

(f) calculating a ratio of ~~(a)~~ the amount of the first type of thyroglobulin measured in (d) to the amount of total thyroglobulin; or ~~(b)~~ the amount of second type of thyroglobulin measured in (e) to the amount of total thyroglobulin; and

(g) determining the malignancy of a thyroid tumor by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living

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body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant in any of the following cases (i) to (iv),

(i) when the calculated ratio is significantly higher ~~or lower~~ than that of the reference fluid sample of the normal thyroid and is significantly higher ~~or lower~~ than that of the reference fluid sample of the benign thyroid,

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid,

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, or

(iv) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid.

Claim 72 (Currently amended): A method for determining malignancy of a thyroid tumor comprising the steps of:

- (a) adding to a sample originating from a living body:
 - (i) a specific lectin or a specific antibody capable of binding to a specific

structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin, and

- (ii) an anti-thyroglobulin antibody-2 capable of binding to the two types of thyroglobulin, but not capable of binding to the thyroglobulin to which the specific lectin or the specific antibody is already bound,

to form a first conjugate which is a conjugate of the specific lectin or the specific antibody with the first type of thyroglobulin, and a second conjugate which is a conjugate of the anti-thyroglobulin antibody-2 with the second type of thyroglobulin;

- (b) separating the first conjugate and the second conjugate; and

- (c) measuring an amount of the first type of thyroglobulin on the basis of the first conjugate content; and

- (d) measuring an amount of the second type of thyroglobulin on the basis of the second conjugate content;

- (e) calculating a ratio of ~~(a)~~ the amount of the first type of thyroglobulin measured in (c) to the amount of total thyroglobulin; or ~~(b)~~ the amount of second type of thyroglobulin measured in (d) to the amount of total thyroglobulin; and

- (f) determining the malignancy of a thyroid tumor by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

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wherein the sample is determined to be malignant in any of the following cases (i) to (iv),

(i) when the calculated ratio is significantly higher ~~or lower~~ than that of the reference fluid sample of the normal thyroid and is significantly higher ~~or lower~~ than that of the reference fluid sample of the benign thyroid,

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid,

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, or

(iv) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid.

Claim 73 (Currently amended): A method for determining malignancy of a thyroid tumor comprising the steps of:

(a) dividing a fluid sample originating from a living body into a first portion and a second portion;

(b)(i) adding to the first portion a specific lectin or a specific antibody capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of

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binding to a sugar chain of a second type of thyroglobulin,

to permit the precipitation of a conjugate of the first type of thyroglobulin with the specific lectin, or the first type of thyroglobulin with the specific antibody;

- (ii) separating the precipitated conjugate from the second type of thyroglobulin; and
- (iii) measuring an amount of the second type of thyroglobulin of the separated part first portion by adding a first anti-thyroglobulin antibody capable of binding to both types of the thyroglobulin; and
- (c)(i) measuring an amount of the total thyroglobulin of the second portion; and
- (ii) determining an amount of the first type of thyroglobulin from the difference between an amount of the total thyroglobulin and the amount of the second type of thyroglobulin obtained in step (b)(iii);
- (d) calculating a ratio of ~~(a)~~ the amount of the first type of thyroglobulin measured in (c)(ii) to the amount of total thyroglobulin; or ~~(b)~~ the amount of second type of thyroglobulin measured in (b)(iii) to the amount of total thyroglobulin; and
- (e) determining the malignancy of a thyroid tumor by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant in any of the following cases (i) to (iv),

(i) when the calculated ratio is significantly higher ~~or lower~~ than that of the reference fluid

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sample of the normal thyroid and is significantly higher ~~or lower~~ than that of the reference fluid sample of the benign thyroid,

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid.

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, or

(iv) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid.

Claim 74 (Currently amended): A method for determining malignancy of a thyroid tumor comprising the steps of:

(a) adding to a fluid sample originating from a living body, a specific lectin or a specific antibody capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin; then

(b) adding to the sample a first antibody, capable of binding to both types of thyroglobulin, to form a first conjugate which is a conjugate of the first antibody with the first type of thyroglobulin and with the specific lectin or the specific antibody, and a second conjugate which

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is a conjugate of the first antibody with the second type of thyroglobulin;

(c) separating the first conjugate and the second conjugate; and

(d) measuring an amount of the first type of thyroglobulin on the basis of the first conjugate content; and

(e) measuring an amount of the second type of thyroglobulin on the basis of the second conjugate content;

(f) calculating a ratio of ~~(a)~~ the amount of the first type of thyroglobulin measured in (d) to the amount of total thyroglobulin; or ~~(b)~~ the amount of second type of thyroglobulin measured in (e) to the amount of total thyroglobulin; and

(g) determining the malignancy of a thyroid tumor by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant in any of the following cases (i) to (iv),

(i) when the calculated ratio is significantly higher ~~or lower~~ than that of the reference fluid sample of the normal thyroid and is significantly higher ~~or lower~~ than that of the reference fluid sample of the benign thyroid,

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid,

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(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, or

(iv) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid.

Claim 75 (Currently amended): A method for determining malignancy of a thyroid tumor comprising:

(a) dividing a fluid originating from a living body into a first portion and a second portion;

(b)(i) adding to the first portion a specific lectin or a specific antibody capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin,

to form a conjugate of the first type of thyroglobulin with the specific lectin or the specific antibody; then

(ii) adding to the first portion an antibody-2 capable of binding to the two types of thyroglobulin, but not capable of binding to the thyroglobulin to which the specific lectin or the specific antibody is already bound, to form a conjugate of the second type of thyroglobulin with the antibody-2; and

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- (iii) measuring the amount of the second type of thyroglobulin on the basis of the measurement of the second type of thyroglobulin with antibody-2 conjugate formed in step (b)(ii); and
- (c)(i) measuring an amount of the total thyroglobulin of the second portion; and
- (ii) determining an amount of the first type of thyroglobulin from the difference between an amount of the total thyroglobulin and the amount of the second type of thyroglobulin obtained in step (b)(iii);
- (d) calculating a ratio of ~~(a)~~ the amount of the first type of thyroglobulin measured in (c)(ii) to the amount of total thyroglobulin; or ~~(b)~~ the amount of second type of thyroglobulin measured in (b)(iii) to the amount of total thyroglobulin; and
- (e) determining the malignancy of a thyroid tumor by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant in any of the following cases (i) to (iv).

(i) when the calculated ratio is significantly higher ~~or lower~~ than that of the reference fluid sample of the normal thyroid and is significantly higher ~~or lower~~ than that of the reference fluid sample of the benign thyroid,

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign

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thyroid.

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, or

(iv) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid.

Claim 76 (Currently amended): The method of any one of claims ~~51, 53, 56~~, 59 and 68-75, wherein said specific antibody is one reactive with an Lewis type sugar chain.

Claim 77 (Currently amended): The method according to any one of claims ~~51, 53, 56~~, 59 and 68-75, wherein the sugar chain with the specific structure is one found in thyroglobulin which is produced by a carcinoma cell.

Claim 78 (New): A method for determining malignancy of a thyroid tumor comprising the steps of:

(a) adding to a fluid sample originating from a living body;

(i) a specific pectin or a specific antibody capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a

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second type of thyroglobulin ,

(ii) a first anti-thyroglobulin antibody, capable of binding to both types of thyroglobulin, and

(iii) a second anti-thyroglobulin antibody, capable of binding to the two types of thyroglobulin, but not capable of binding to the thyroglobulin to which the specific pectin or the specific antibody is already bound,

to form a first conjugate which is a conjugate of the first anti-thyroglobulin antibody with the first type of thyroglobulin and with the specific pectin or the specific antibody, and a second conjugate which is a conjugate of the first anti-thyroglobulin antibody with the second type of thyroglobulin and the second anti-thyroglobulin antibody;

(b) measuring an amount of the first type of thyroglobulin; and

(c) measuring an amount of the second type of thyroglobulin;

(d) calculating a ratio of the amount of the first type of thyroglobulin measured in (b) to the amount of total thyroglobulin; or the amount of second type of thyroglobulin measured in (c) to the amount of total thyroglobulin; and

(d) determining the malignancy of a thyroid tumor by comparing the calculated ratio with a corresponding predetermined ratio from a reference fluid sample originating from a living body having a normal thyroid or a benign thyroid;

wherein the sample is determined to be malignant in any of the following cases (i) to (iv),

(i) when the calculated ratio is significantly higher than that of the reference fluid

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sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid,

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid,

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, or

(iv) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid.